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Published in:
FASEB Journal

DOI:
10.1096/fj.201901275R

Publication date:
2019

THE FASEB JOURNAL • RESEARCH • www.fasebj.org

Telomere length tracking in children and their parents: implications for adult onset diseases

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ABSTRACT: Adults with comparatively short or long leukocyte telomere length (LTL) typically continue to display comparatively short or long LTL throughout life. This LTL tracking stems from the inability of person-to-person variation in age-dependent LTL shortening during adulthood to offset the wide interindividual LTL variation established prior to adult life. However, LTL tracking in children is unstudied. This study aimed to examine LTL shortening rates and tracking in children and their parents. Longitudinal study in children (n = 67) and their parents (n = 99), whose ages at baseline were 11.4 ± 0.3 and 43.4 ± 0.4 yr, respectively. LTL was measured by Southern blotting at baseline and ~14 yr thereafter. LTL displayed tracking in both children [intraclass correlation coefficient (ICC) = 0.905, $P < 0.001$] and their parents (ICC = 0.856, $P < 0.001$). The children's rate of LTL shortening was twice that of their parents (40.7 ± 2.5 bp/yr; 20.3 ± 2.1 bp/yr, respectively; $P < 0.0001$). LTL tracking applies not only to adulthood but also to the second decade of life. Coupled with previous work showing that the interindividual variation in LTL across newborns is as wide as in their parents, these findings support the thesis that the LTL-adult disease connection is principally determined before the second decade of life, perhaps mainly at birth.—Benetos, A., Verhulst, S., Labat, C., Lai, T.-P., Girerd, N., Toupance, S., Zannad, F., Rossignol, P., Aviv, A. Telomere length tracking in children and their parents: implications for adult onset diseases. FASEB J. 33, 14248–14253 (2019). www.fasebj.org

KEY WORDS: aging · telomere attrition · leukocytes · terminal restriction fragments

Converging lines of evidence suggest that, as expressed in leukocytes, telomere length (TL) plays a causal role in atherosclerotic cardiovascular disease (CVD) and major cancers. Individuals with comparatively short leukocyte TL (LTL) display propensity for CVD (1, 2), whereas their peers with comparatively long LTL display propensity for major cancers (3, 4). Mendelian randomization of LTL-associated single nucleotide polymorphisms have inferred that these LTL-disease associations are causal (5–8). Learning the determinants of LTL is therefore of fundamental relevance to understanding the role of telomeres in the 2 disease categories that ultimately afflict the majority of contemporary humans.

The individual's LTL is shaped by LTL dynamics (*i.e.*, LTL at birth and age-dependent LTL shortening thereafter). General features of LTL dynamics during the human life course are now known. First, LTL is highly variable across individuals from birth onwards (9–11). Second, person-to-person variation in the rate of LTL shortening during adulthood (12) is usually insufficient to overcome interindividual variation in LTL that were established prior to adulthood. Therefore, individuals entering adult life display LTL tracking (*i.e.*, individuals with comparatively short or long LTL typically maintain their short or long LTL throughout their remaining life course) (10). Notably, cross-sectional studies indicate a rapid loss of TL in the first decades of life (13, 14). Little is known, however, based on longitudinal studies, about interindividual variation in the rate of LTL shortening and tracking during the first 2 decades of life. To fill this knowledge gap, we studied longitudinal measurements, LTL shortening rates, and tracking in children and their parents.

ABBREVIATIONS: CI, confidence interval; CVD, cardiovascular disease; ICC, intraclass correlation coefficient; LTL, leukocyte telomere length; TL, telomere length

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doi: 10.1096/fj.201901275R
This article includes supplemental data. Please visit *http://www.fasebj.org* to obtain this information.

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MATERIALS AND METHODS

The cohort

The Suivi Temporaire Annuel Non-Invasif de la Sante des Lorrains Assures Sociaux (STANISLAS). Study is a single-center, familial longitudinal study comprising 1006 families (4295 participants) from the Nancy region of France (ClinicalTrials.gov identifier: NCT01391442). The study and its goals have been previously described by Ferreira *et al.* (15). For this telomere project, we identified in the STANISLAS biorepository blood samples, donated between May 1998 and February 2001 (baseline) and samples donated between July 2011 and June 2016 (follow-up) by children, aged <14 yr at baseline, and their parents.

LTL measurements

DNA were obtained from buffy coats (baseline samples) and whole blood (follow-up samples) using a salting-out method as previously described by Miller *et al.* (16). DNA samples passed an integrity test using a 1% (w/v) agarose gel before LTL measurements performed by Southern blotting of the terminal restriction fragments, as previously described by Kimura *et al.* (17). Briefly, DNA samples were digested (37°C) overnight with restriction enzymes Hinf I and Rsa I (Roche, Basel, Switzerland). Digested DNA samples and DNA ladders were resolved on 0.5% (wt/vol) agarose gels. After 23 h, the DNA was depurinated, denatured, neutralized, and transferred onto a positively charged nylon membrane (Roche) using a vacuum blotter (Bio-Rad, Hercules, CA, USA). Membranes were hybridized at 65°C with the DIG-labeled telomeric probe, after which the probe was detected by the DIG luminescent procedure (Roche) and exposed on X-ray film. The interassay coefficient of variation for the duplicate measurements (on different gels) was 1.2%, and the intraclass correlation coefficient (ICC) was 0.95 [95% confidence interval (CI): 0.79–1; $n = 183$].

Statistical analysis

Data were analyzed using general linear mixed models in R (18), using the packages lme4 and lmerTest. As random effects, we included family or individual identity. We used the ICC, estimated from models that included age, to estimate the extent to which individuals at different ages differed consistently in LTL from other individuals within their class (children or parents). Age varied between and within individuals because they were sampled twice. To investigate whether LTL shortening in parents and their children (*i.e.*, older and younger individuals) was age dependent, we transformed age at sampling into 2 variables (*i.e.*, the average age at which each individual was sampled and the deviation from the average age). For example, when an individual was sampled at 25 and 35 yr of age, average age = 30 for both samples, whereas Delta age = −5 for the baseline sample, and Delta age = +5 for the follow-up sample. Thus, the slope of Delta age represents the (longitudinal) rate of LTL shortening within individuals, whereas the slope of average age represents the cross-sectional rate of LTL shortening.

To avoid confounding heritability estimates with correlated ages of parents and their children, we used LTL estimates corrected for age by calculating residuals. A midparent LTL estimate was calculated after averaging the residuals of both parents for families, where LTL of both parents was known. These LTL values were transformed to standard normal distributions prior to analysis for parents and children separately.

Female patients have a longer LTL than male patients (19), but capturing this difference requires a larger data set than in the present study (20). Hence, LTL was not corrected for sex in our

TABLE 1. *Age and LTL values at baseline and follow-up visits in children and their parents*

Variable	Children	Parents	*P*
Cohort (*n*)	67	99	
Women (%)	57	53	0.59
Age BL (yr)	11.36 ± 0.28	43.41 ± 0.37	<0.0001
Delta age FU (yr)	14.02 ± 0.13	13.53 ± 0.09	0.004
LTL BL (kb)	8.32 ± 0.08	7.42 ± 0.06	<0.0001
LTL FU (kb)	7.75 ± 0.08	7.15 ± 0.06	<0.0001
LTL shortening (bp/yr)	40.7 ± 2.5	20.3 ± 2.1	0.001

Values are means ± SEM. BL, baseline; FU, follow-up.

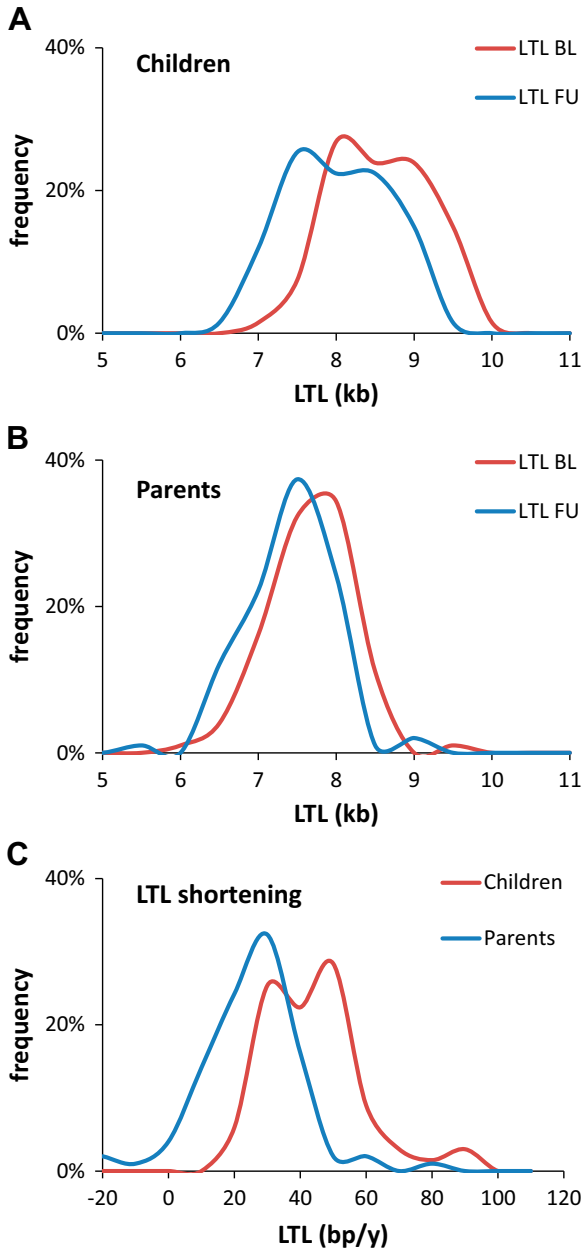

Figure 1. LTL distribution at baseline and follow-up visits in children (*A*) and their parents (*B*), and LTL shortening (parents and children) (*C*). BL, baseline; FU, follow-up.

analyses, but its inclusion made negligible difference in the results.

Comparisons in **Table 1** were performed using the Mann-Whitney and χ^2 tests, and data are presented as means ± SEM.

RESULTS

General characteristics of participants are displayed in Table 1, and a breakdown of the 57 families by parents and siblings is provided in Supplemental Fig. S1.

LTL dynamics in children and parents

LTL was longer by 906 ± 83 base pairs (bp; $t = 10.88$, $P < 0.001$) at baseline and by 622 ± 80 bp ($t = 7.75$, $P < 0.001$) at follow-up in children than their parents (Table 1). **Figure 1** shows the LTL distribution at baseline and follow-up in children (Fig. 1*A*) and their parents (Fig. 1*B*), and LTL shortening in both populations (Fig. 1*C*). LTL at follow-up was shorter than at baseline by 572 ± 34 bp in the children ($t = 16.93$, $P < 0.001$) and by 275 ± 30 in the parents ($t = 9.13$, $P < 0.001$). These findings indicate that LTL shortening was faster in children than in parents (children, 40.7 ± 2.5 bp/yr, $t = 16.22$, $P < 0.001$; parents, 20.3 ± 2.1 bp/yr, $t = 9.47$, $P < 0.001$; interaction, $t = 6.21$, $P < 0.001$; Table 1 and Fig. 1*C*).

Tracking

We quantified differences in LTL across individuals within their class (children or parents) at different ages using the ICC estimated from models that included age (**Tables 2 and 3**). For children, the ICC = 0.905 (95% CI: 0.588–1; $P < 0.001$; **Fig. 2*A***), whereas for parents the ICC = 0.856 (95% CI: 0.601–1; $P < 0.001$; Fig. 2*B*). This denotes high and indistinguishable consistency in tracking of age-dependent LTL between children and parents.

Heritability of LTL

The regression of the children LTL on midparent LTL, including family identity as random effect, yielded an estimated narrow sense heritability of 0.35 ± 0.13 ($P = 0.01$; **Tables 4**). Regression on paternal LTL yielded an estimate of 0.80 (*i.e.*, 2 times the coefficient in **Table 5**), whereas an estimate based on regression on maternal LTL yielded an estimate of 0.35 (**Table 4**). Only a minority of the families had more than 1 child in these analyses (see sample sizes in Supplemental Fig. S1); hence, we attach little value to the variance explained by family identity as random effect (exclusion of family identity had little effect on the estimates). In addition, LTL correlation between parents and children observed in this study (see also Supplemental Fig. S2) might also stem from shared environment as well as heritability. However, LTL was poorly correlated between parents ($P = 0.36$), suggesting a negligible environmental effect on LTL, at least in the parents (Supplemental Fig. S3).

TABLE 2. *LTL in relation to age*

Variable	Estimate ± SE	df	*T*	*P*
Children				
Intercept	9.290 ± 0.576	65.0	16.12	<0.0001
Average age	−0.0681 ± 0.0311	65.0	−2.19	0.032
Delta age	−0.0407 ± 0.0024	65.0	−17.09	<0.0001
Parents				
Intercept	8.174 ± 0.716	102.46	11.41	<0.0001
Average age	−0.0181 ± 0.0142	102.57	−1.28	0.204
Delta age	−0.0203 ± 0.0022	98.37	−9.19	<0.0001

Children ($n = 134$ observations on 67 individuals). Parents ($n = 203$ observations on 104 individuals). Average age is the mean age at which individuals were sampled. For each sampling point, Delta age is the difference between average age and age at sampling. Delta age yields the estimate for longitudinal effects of age on LTL. Average age is not significant for parents, but is retained in the model for consistency.

TABLE 3. *LTL in relation to age: random effect and variance*

Random effect	Variance
Children	
Individual identity	0.361
Residual	0.038
Parents	
Individual identity	0.268
Residual	0.045

DISCUSSION

The key findings of this longitudinal evaluation of LTL dynamics in children and their parents are as follows: *1*) the rate of age-dependent LTL shortening is much faster during the second decade than in adulthood (from third decade onwards), and *2*) LTL tracking is a phenomenon that applies not only to adults but also to children during their second decade of life. In addition, we confirmed LTL heritability, observed in previous studies (21, 22), but the small sample size might explain lower heritability found in this study than observed previously. For the same reasons, there was no detectable heritability of LTL shortening in this study (unpublished results).

Given the age range of children participating in this study, we do not know at present whether the LTL tracking phenomenon also covers the first decade of life, but we consider this likely. The implications of our findings are considerable because they suggest that LTL trajectory during adulthood is largely determined before the second decade of life (*i.e.*, at birth and during the first decade of life). Moreover, based on cross-sectional data derived from our previous study of LTL in newborns and their parents (9), our work using skeletal muscle TL as a reference of early life TL (11, 23) and longitudinal data on children and parents in this study, it is clear that LTL shortening during the first decade is much faster than during the second decade of life (details are elaborated under Supplemental Data). Such findings confirm theoretical considerations of LTL dynamics due the expansion of the hematopoietic system during somatic growth (24), which jointly with empirical data indicate that LTL

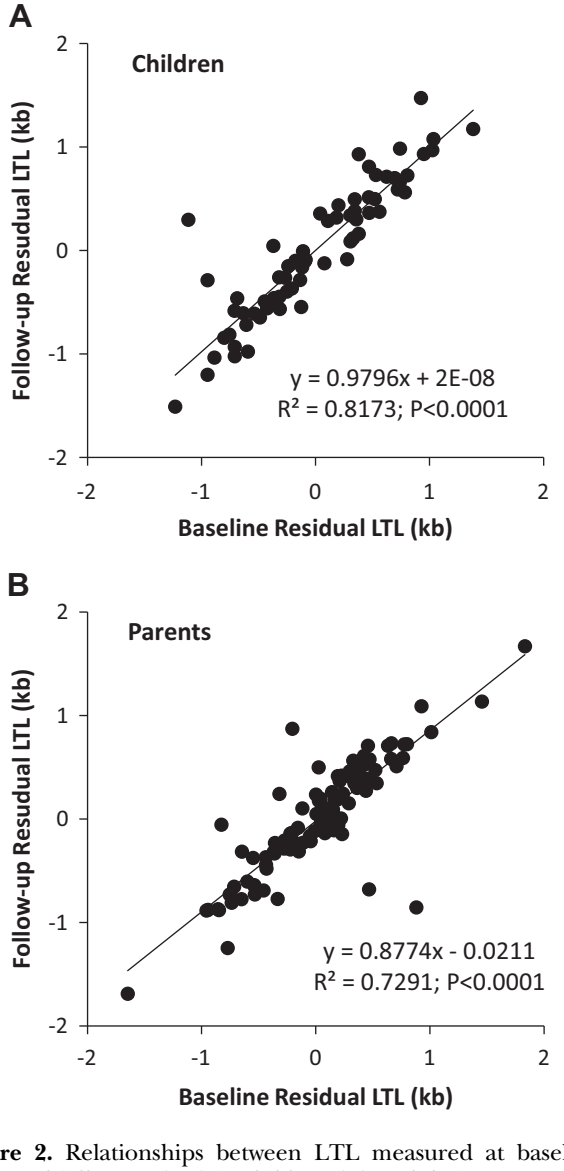

Figure 2. Relationships between LTL measured at baseline (BL) and follow-up (FU) in children (*A*) and their parents (*B*).

shortening during the first decade of life is inversely related to age and amounts to ~1 kb. This body of population-based telomere research underscores the importance of birth LTL and LTL shortening during the first decade as determinants of LTL throughout the life course.

TABLE 4. *LTL inheritance: explanatory variable*

Variable	Estimate ± SE	df	*T*	*P*
Intercept	−0.050 ± 0.133	45.78	0.38	0.73
Midparent LTL	0.349 ± 0.134	46.15	2.61	0.012
Paternal LTL				
Intercept	−0.055 ± 0.128	44.16	0.43	0.67
Paternal LTL	0.399 ± 0.132	44.97	3.02	0.004
Maternal LTL				
Intercept	−0.061 ± 0.130	51.48	0.47	0.64
Maternal LTL	0.177 ± 0.128	53.43	1.38	0.17

Regression of LTL of children on parental LTL. LTL was corrected for age and transformed to a standard normal distribution for parents and children separately. Children LTL regressed on midparent LTL (n = 56 children from 48 families). Children LTL regressed on paternal LTL (n = 58 children from 50 families). Children LTL regressed on maternal LTL (n = 64 children from 54 families).

TABLE 5. *LTL inheritance: random effect and variance*

Random effect	Variance
Midparent LTL	
Family identity	0.529
Residual	0.356
Paternal LTL	
Family identity	0.492
Residual	0.362
Maternal LTL	
Family identity	0.608
Residual	0.346

Coupled with recent Mendelian randomization analyses that infer a causal role of LTL in CVD and cancer (5–8), the LTL tracking phenomenon provides further impetus to understand intrinsic mechanisms that determine the length of human telomeres in the newborn and the impact of nonheritable parameters on LTL dynamics throughout the life course but particularly *in utero* and early extrauterine life. This is relevant, given that TL dynamics in the hematopoietic system, as expressed in LTL dynamics, and not, for instance, skeletal muscle TL, explains the role of telomeres in atherosclerotic CVD (25).

Finally, the concept that absolute LTL is a biomarker (bioclock) of human biologic aging has dominated telomere population research as no other concept has, and it remains stubbornly popular despite overwhelming evidence that suggests otherwise (9–11, 25–27). Classically, a bioclock is a marker reflecting the loss of function or substance with passing time. The extension of the LTL tracking from adulthood to childhood and possibly to birth, coupled with wide interindividual variation in LTL in newborns (9), provide the strongest evidence refuting this concept. Indeed, even if LTL shows a progressive attrition with age, LTL absolute value at a given age reflects essentially TL at birth and TL attrition during the first decade and much less TL loss after childhood. Longitudinal studies that determine the rate of age-dependent LTL attrition might provide insight into biologic pathways linked to aging, but such information is rudimentary at present. In conclusion, the ramifications of LTL tracking from the second decade onward are sweeping because they point to a lifelong influence of LTL at birth and perhaps early childhood on disease risk during adulthood. It is imperative, therefore, that intensive research is undertaken to understand the root causes of the interindividual LTL variation at birth and the first decade of life. FJ

ACKNOWLEDGMENTS

This work was supported by the regional project Contrats de Plan État-Région (CPER)–Innovations Technologiques, Modélisation et Médecine Personnalisée (ITM2P) 2015–2020, the French PIA Project Lorraine Université d'Excellence (ANR-15-IDEX-04-LUE), and the Investments for the Future

Program under Grant ANR-15-RHU-0004. The Suivi Temporaire Annuel Non-Invasif de la Sante des Lorrains Assures Sociaux (STANISLAS) study was sponsored by Nancy Centre Hospitalier Régional Universitaire (CHRU), Université de Lorraine. Abraham Aviv research was supported by U.S. National Institutes of Health (NIH) Grants R01HL116446 and R01HL13840 (National Heart, Lung, and Blood Institute), R01HD071180 (Eunice Kennedy Shriver National Institute of Child Health and Human Development), and Norwegian Institute of Public Health Grants 262700 and 262043. The STANISLAS cohort study is declared on ClinicalTrials.gov under the identifier NCT01391442. The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

A. Benetos, S. Toupance, F. Zannad, P. Rossignol, and A. Aviv designed research; T.-P. Lai, N. Girerd, and S. Toupance performed research; T.-P. Lai contributed new reagents or analytic tools; S. Verhulst and C. Labat analyzed data; A. Benetos and A. Aviv drafted the initial manuscript, and reviewed and revised the paper; S. Verhulst, C. Labat, T.-P. Lai, N. Girerd,S. Toupance, F. Zannad, and P. Rossignol revised the paper for important intellectual content; and all authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

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Received for publication May 24, 2019.
Accepted for publication September 17, 2019.